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RESEARCH PAPER

Comprehensive assessment of salt-alkali tolerance of different soybean varieties at the maturation stage

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Abstract

Soil salinization is a significant global challenge impacting food security and arable land safety. Biological amelioration for the development and utilization of salt-alkali land has gained attention in recent years but the breeding and selection of salt-alkali-tolerant crop varieties remain a challenge. Cultivated soybeans are moderately salt-tolerant crops; however, a precise threshold for salt-alkali conditions has not been determined and varies by genotype. Therefore, this study evaluated the salt-alkali tolerance of 15 different soybean varieties using correlation, principal component, membership function, and cluster analyses. The average soybean indices decreased with increasing salt-alkali stress concentrations. Under severe salt-alkali stress, the coefficients of variation for grain yield and biomass were significantly higher than those under mild salt-alkali stress. Plant height and node number were significantly positively correlated, whereas bottom pod height was significantly negatively correlated with branch number, effective pod number, and grain number per plant. Node and grain numbers per plant were significantly positively correlated. Branch number, effective pod number, grain number per plant, and hundred-grain weight were significantly and positively correlated. Effective pod number was significantly positively correlated with grain number per plant, biomass, and yield. Grain number per plant exhibited a significant negative correlation with hundred-grain weight. Biomass and yield were significantly positively correlated. The salt-alkali tolerance of 15 soybean varieties ranged from strong weak as follows: Cangdou 1438, 0734, 1426, 1418, 1453, 1327, 1846, 1412, 1817, 1301, 1821, 1819, 1815, 1814, and 1850. Salt-alkali-sensitive and salt-alkali-tolerant varieties were successfully identified.

Keywords

Agronomic traits, maturation stage, salt-alkali tolerance, soil salinization, soybean, yield indices

Introduction

Soil salinization poses a global challenge, resulting in the worldwide depletion of land resources. Saline soils cover approximately 8.0 billion hectares of land globally, accounting for 6% of the total land area (Munns 2005). In China, the total area of various types of saline soils is approximately 99.13 million hectares, representing 1.03% of the country's total land area. Soil salinization in arable land has been reported in 6.62% of the total arable land area (Yang and Wang 2015). Soil salinization contributes to soil degradation and significantly hampers agricultural production and economic development in China. It directly affects the nation's food security and arable land safety (Shi et al. 2015; Liu and Li 2019). Therefore, improving and utilizing salt-alkali land would be beneficial for the sustainable development of agriculture

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and rural areas in China. Biological amelioration, as a cost-effective, low-scale, long-lasting, and ecologically friendly soil improvement measure, has gained increasing attention in recent years (Luo et al. 2001). Therefore, the breeding and selection of salt-alkali tolerant crop varieties are significant for the development and utilization of salt-alkali land.

Cultivated soybeans fall within the category of moderately salt-tolerant crops, with a soil salinity threshold of approximately 5 dS/m (Ayers and Westcot 1989; Hosseini et al. 2002; Guo and Weng 2004). However, a precise threshold for salt-alkali conditions has not been determined, and soybean salt-alkali tolerance varies by genotype. Existing non-biological stress identification methods using hydroponics or field trials have been employed for most soybean salt-alkali tolerance assessments. Shao et al. (1986) determined the salt-alkali tolerance of more than 1,700 soybean resources through experiments in which they watered them with a solution containing saline groundwater (primarily NaCl) and tap water, resulting in the selection of seven salt-tolerant varieties across the full growth cycle. Subsequently, they used 210 mmol·L^{-1} NaCl to screen 941 soybean germplasms during the germination and seedling stages and identified 21 level-1 salt-tolerant varieties (Gai 2007). Na et al. (2009) selected five highly salt-tolerant varieties, seven highly alkali-tolerant varieties, and two varieties exhibiting both high alkali and high salt tolerance from 100 seeds treated with 110 mmol·L^{-1} NaCl or 37.5 mmol·L^{-1} Na_2CO_3. Zhang et al. (2018) conducted salt-alkali tolerance assessments of 161 soybean resources in the Huang–Huai–Hai region during the seedling and field stages and identified 12 soybean varieties suitable for cultivation in mildly saline-alkali areas of the Yellow River Delta. Moreover, researchers at various institutions, including the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Yan'an University, and Shandong Crop Germplasm Resource Center, screened the salt-tolerant soybean resources but on a smaller scale (Tuyen et al. 2010; Pu et al. 2018; Liu et al. 2020).

To date, research on salt-alkali tolerance assessment of soybean germplasm has mainly focused on the germination and seedling stages under indoor conditions, whereas studies on salt-alkali tolerance during the maturation and harvest stages under field conditions are limited. Establishment of defined field conditions during the assessment of salt-alkali tolerance of soybeans during the maturation stage would enable the replication of real-world stress conditions, providing references for practical production. In this study, we conducted field experiments to analyze the agronomic traits and yield indices of 15 soybean varieties during the maturation stage. We used weighted membership function analysis to evaluate and categorize the salt-alkali tolerance of different soybean varieties, and we identified and selected soybean germplasm resources with strong salt-alkali tolerance and high yield potential. Our results provide insights into the effective development and utilization of salt-alkali land.

Materials and methods

Soybean varieties

Fifteen soybean varieties were selected as the experimental materials. The serial numbers, names, and sources are listed in Table 1.

Table 1. Soybean varieties.

SN	Name	parent
1	Cangdou 1850	Cangdou 09Y1/Jidou 1507
2	Cangdou 1846	Cangdou 09Y1/Lu 96150
3	Cangdou 1821	Cangdou 15/Wandou 38
4	Cangdou 1819	Cangdou 13/Shanning 21
5	Cangdou 1817	Hedou 12/Yandou 9
6	Cangdou 1815	Cangdou 13/Shidou 14
7	Cangdou 1814	Cangdou 10/Han 1325
8	Cangdou 1453	Zhongzuo02-958/Jidou 21
9	Cangdou 1438	Jidou 12/Lu 96451
10	Cangdou 1426	Hedou 12/Fendou 94
11	Cangdou 1418	Cangdou 11/Zhongzuo J8012
12	Cangdou 1412	Cang M0805/Zhongzuo 984-2
13	Cangdou 1327	Hedou 12/Ji 09B2
14	Cangdou 1301	Cangdou 11/Lu 97013
15	Cangdou 0734	Jidou12/Zhongzuo 122

Soybean varieties used in the experiments were all sourced from Cangzhou Academy of Agriculture and Forestry Sciences.

Experimental design

The experiments were conducted from June to October 2021 and 2022 in Changguo Town, Huanghua City, Cangzhou, Hebei Province (38°16'52.8"N, 117°17'4.5"E). Two types of experimental fields were established: mildly salt-alkali land (pH 8.11) and severely salt-alkali land (pH 8.95). The physicochemical properties of the soils are listed in Table 2. A randomized block design was employed with plot sizes of 18 m^2, row spacing of 0.5 m, plant spacing of 0.09 m, and 1 m spacing between adjacent plots. Three replicate experiments were performed for each variety, with 2 m buffer rows planted around each experimental field. Prior to sowing, the land was prepared and leveled, and a compound fertilizer [w(N): w(P_2O_5): w(K_2O) = 15:15:15] was applied at a rate of 225 kg/hm^2 as a base fertilizer. Only fully uniformly sized seeds were selected from each variety for sowing. Throughout the soybean growth period, regular inter-row tillage was applied for weed control, irrigation, and fertilization. Two rounds of inter-row tillage for weed control were conducted during the seedling and early flowering stages, and disease and pest control measures were implemented as needed according to field conditions. Harvesting was performed at maturity and irrigation was not carried out during the entire growth period.

Table 2. Physicochemical properties of the plots.

Plot (depth)	ρ(N) (g·kg^{-1})	ρ(P) (g·kg^{-1})	ρ(K) (g·kg^{-1})	ρ(Na) (g·kg^{-1})	ρ(Cl) (g·kg^{-1})	ρ(CO_3+ HCO_3) (g·kg^{-1})	ρ(SO_4) (g·kg^{-1})	pH
Mildly salt-alkali land (0–40 cm)	0.67	2.19	0.03	1.15	0.5	0.13	0.48	8.11
Severely salt-alkali land (0–40 cm)	0.58	2.13	0.06	1.80	1.75	0.49	1.39	8.95

Measurement parameters and methods

Two days prior to harvesting, five soybean plants with consistent growth were selected consecutively from each plot for indoor examination. Plant height was measured, and data on the main stem nodes, effective branches, pod number per plant, grain number per plant, and hundred-grain weight were recorded. Subsequently, the plants were separated into roots, stems, pods, and other organs (during the maturation stage, most leaves and petioles had fallen off and were negligible). The plants were placed in an oven at 105 °C for 30 min and then dried at 80 °C until they reached a constant weight. The dry weight of each organ was measured, and the total dry weight of the plants was calculated. After harvesting, the actual yield in the middle four rows of each plot (10 m^2) was measured. The grains were threshed and air-dried, and the grain yield of each plot was determined in hectares (kg/hm^2).

Statistical analysis

The data were organized and analyzed using Excel 2010 to calculate the means and standard deviations. SPSS (version 23.0; IBM Corp., Armonk, NY, USA) was used to analyze the correlations and perform principal component analysis of the data. DPS 7.05 was employed for the cluster analysis of comprehensive evaluation values (*D*), and membership function analysis was performed to evaluate soybean salt-alkali tolerance.

Salt tolerance index (*Ist*) is the ratio of measurement values under severe salt stress to those under mild salt stress.

Membership function value $U(X_j) = (X_j - X_{min})/(X_{max} - X_{min})$, where i is the score of the trait measurement of the *j*th factor for soybeans, X_{min} is the minimum score for the *j*th factor, and X_{max} is the maximum score for the *j*th factor.

Factor weight W_j:

$$W_j = \frac{P_j}{\sum_{j=1}^{m} P_j}, j = 1,2,\bullet\bullet\bullet,n \quad (1)$$

where W_j is the importance of the *j*th factor among all common factors and P_j is the contribution rate of each variety to the *j*th factor.

Comprehensive evaluation *D*:

$$D_i = \sum_{j=1}^{m} [U(X_j)] \times W_j, j = 1,2,3,\bullet\bullet\bullet \quad (2)$$

where D_i is the comprehensive evaluation value of soybean salt-alkali tolerance at the maturation stage.

Degree of plant growth inhibition by salt-alkali stress. This index was calculated as the ratio of the difference between the measured values under salt-alkali stress to those under control conditions.

Results

Effect of salt-alkali stress on soybean growth at the maturation stage

Under severe salt-alkali stress conditions, all soybean indices except for bottom pod height decreased compared to those under mild salt-alkali stress (Table 3). The extent of inhibition as a consequence of stress varied for each index, ranking from highest to lowest as follows: yield (15.66%); branch number (13.33%); biomass (12.65%); effective pod number (12.61%); grain number per plant (12.14%); plant height (8.12%); and node number (5.78%). The hundred-grain weight also decreased under severe salt-alkali stress by 5.59% compared to that under mild salt-alkali stress. Notably, different soybean varieties showed varying levels of salt-alkali tolerance. The coefficient of variation for each trait was higher under severe salt-alkali stress than under mild salt-alkali stress, except for plant height, node number, and grain number per plant. Under severe salt-alkali stress,

Table 3. Growth status of soybeans under mild and severe salt-alkali stress.

Index	Mild salt-alkali stress					Severe salt-alkali stress				
	Min	Max	Mean	SD	COV/%	Min	Max	Mean	SD	COV/%
Plant height	71.90	126.00	86.50	13.72	15.86	63.00	115.00	79.47	11.29	14.21
Bottom pod height	10.90	30.20	20.64	4.26	20.65	10.00	32.10	22.74	4.93	21.69
Node number	14.00	19.00	15.91	1.51	9.48	13.00	18.00	14.99	1.25	8.32
Branch number	0.00	3.00	1.00	0.87	86.99	0.00	3.00	0.87	0.78	89.64
Effective pod number	16.00	45.00	32.88	4.98	15.14	15.00	39.00	28.73	4.98	17.32
Grain number per plant	30.00	89.00	66.41	12.96	19.51	38.00	78.00	58.35	10.82	18.55
Hundred-grain weight	19.20	29.10	23.37	2.99	12.78	18.10	28.60	22.06	2.92	13.22
Biomass	8941.08	10047.46	9768.71	299.95	3.07	7783.63	9544.22	8532.83	510.42	5.98
Yield	2555.68	3148.31	2891.51	177.24	6.13	1864.74	2910.46	2438.57	337.45	13.84

the coefficients of variation for plant yield, biomass, branch number, effective pod number, bottom pod height, and hundred-grain weight were 7.71%, 2.91%, 2.65%, 2.17%, 1.05%, and 0.44% higher, respectively, than those under mild salt-alkali stress. In particular, yield and biomass showed a significantly higher coefficient of variation under severe salt-alkali stress than under mild salt-alkali stress, indicating a considerable difference between these two indices with respect to sensitivity to salt-alkali components.

Effect of salt-alkali stress on soybean yield

Biomass and grain yield serve as crucial indicators for assessing soybean growth. Our results revealed that, under severe salt-alkali stress, soybean biomass and grain yield experienced a significant decrease, with average reductions of 12.65% and 15.68%, respectively, compared to conditions characterized by mild and severe salt-alkali stress (Suppl. material 1: table S1). Under mild salt-alkali stress, the biomass and yield values of eight soybean varieties were higher than the average. The top three varieties in terms of biomass were Cangdou 1453, 1846, and 1817. Regarding the grain yield, the top three varieties were Cangdou 1846, 1453, and 1817; that is, Cangdou 1453 excelled in both categories. Under severe salt-alkali stress, the biomass values of eight soybean varieties were higher than the average, with Cangdou 1438, 1426, and 1453 representing the top three varieties. Similarly, eight varieties had grain yield values higher than average, with Cangdou 1438, 1426, and 1418 representing the top three. The cultivars Cangdou 1438 and 1426 excelled in both categories. In both growth environments, Cangdou varieties 1846, 1817, 1438, 1453, 1418, and 1426 exhibited higher than average biomass values. Similarly, regarding the grain yield, Cangdou varieties 1846, 1817, 1453, 1438, and 1412 demonstrated values that exceeded the average in both growth environments. The salt-alkali tolerance coefficients for these two indices varied among the varieties. The top five varieties were Cangdou 1438, 1426, 1327, and 0734. The coefficients of variation for biomass and yield were 7.71% and 2.91% higher under severe salt-alkali stress than under mild salt-alkali stress, respectively.

Correlation analysis of various indices and salt-alkali tolerance index for soybean yield

We conducted a correlation analysis between the salt-alkali tolerance indices of different soybean varieties and the salt-alkali tolerance index for yield (Suppl. material 1: table S2). The results revealed a significant positive correlation ($P < 0.01$) between plant height and node number and a significant negative correlation ($P < 0.01$) between plant height and branch number. Furthermore, bottom pod height exhibited a significant negative correlation ($P < 0.05$) with node number and a significant negative correlation ($P < 0.01$) with branch number, effective pod number, and grain number per plant. Node number exhibited a significant positive correlation ($P < 0.01$) with grain number per plant, whereas branch number displayed highly significant positive correlations ($P < 0.01$) with effective pod number and grain number per plant and a significant positive correlation ($P < 0.01$) with hundred-grain weight. Effective pod number showed significant positive correlations ($P < 0.01$) with grain number per plant, biomass, and yield. Grain number per plant exhibited a significant negative correlation ($P < 0.01$) with hundred-grain weight. Biomass displayed a significant positive correlation ($P < 0.01$) with yield, with the highest correlation coefficient of 0.935.

Principal component analysis

We conducted a principal component analysis of the nine salt-alkali tolerance evaluation indices for the maturation stage of soybeans and standardized the factor loading matrix; Suppl. material 1: table S3 presents the results. Factors F_1–F_3, with eigenvalues greater than 1, contributed 37.368%, 26.302%, and 15.353%, respectively. The cumulative contribution rate of the three eigenvalues was 79.022%. Subsequently, the nine indices were transformed into three independent comprehensive indices, capturing the majority of the salt-alkali tolerance information of all the original indices. F_1, which contained 37.368% of the original information, mainly comprised hundred-grain weight, biomass, and yield; F_2, which contained 16.302% of the original information, primarily comprised node number, branch number, effective pod number, and grain number per plant; and F_3, which contained 15.353% of the original information, mainly comprised plant and bottom pod heights.

Comprehensive evaluation of salt-alkali tolerance of different soybean varieties under different salt-alkali conditions

The salt-alkali tolerance (D) values ranged from 0.206 to 0.898 (Table 4), with higher D values indicating greater salt-alkali tolerance. The salt-alkali tolerance of the 15 soybean varieties was ranked based on the D values. Based on the maximum distance cluster analysis, the 15 soybean varieties were categorized into five salt-alkali tolerance levels (Fig. 1). Level I, representing the varieties with strong salt-alkali tolerance, included only Cangdou 1438, accounting for 6.67%. Level II, representing the varieties with moderate salt-alkali tolerance, included Cangdou 0734, 1453, 1817, 1418, 1426, 1327, and 1412, accounting for 53.33%. Level III, representing the varieties with salt-alkali sensitivity, comprised Cangdou 1821, 1819, 1301, 1815, and 1814, accounting for 33.33%. Level IV, representing the varieties with high salt-alkali sensitivity, consisted of Cangdou 1850, accounting for 6.67%.

Discussion

In this study, the salt-alkali tolerance of 15 soybean germplasms was evaluated using the salt-alkali tolerance coefficients of different indices, correlation analysis, principal component analysis, and stress tolerance assessment represented by the D value. Based on the magnitude of the D value, the germplasms were classified into four categories: strongly salt-alkali tolerant (one germplasm, Cangdou 1438), moderately salt-alkali tolerant (eight germplasms,

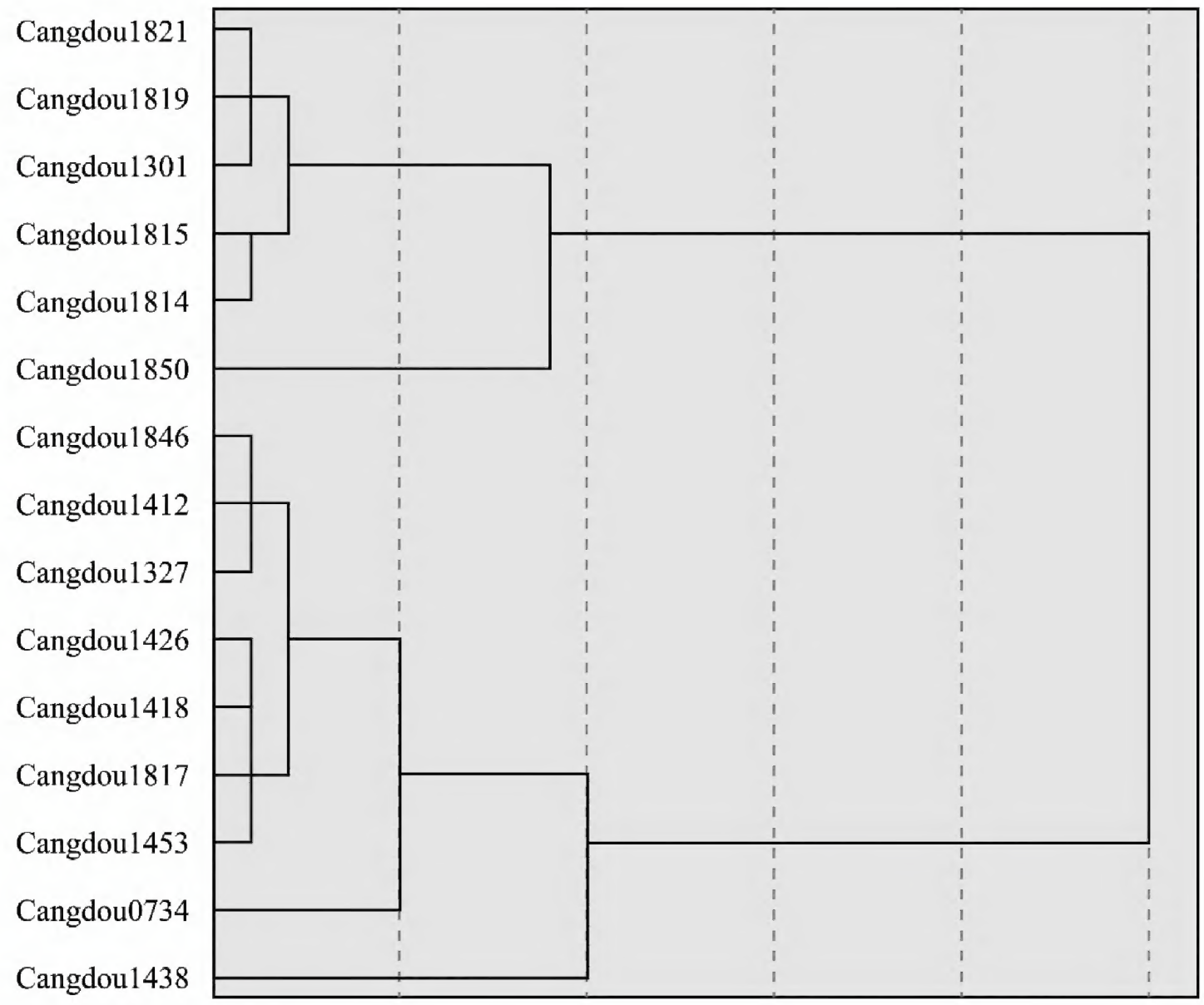

Figure 1. Cluster dendrogram of 15 soybean varieties.

Table 4. Membership function values and comprehensive salt-alkali tolerance evaluation for different varieties at the maturation stage.

Variety	*U1*	*U2*	*U3*	*D*	Ranking
Cangdou 1850	0.000	0.384	0.401	0.206	15
Cangdou 1846	0.648	0.203	0.647	0.589	7
Cangdou 1821	0.396	1.000	0.717	0.458	11
Cangdou 1819	0.370	0.893	0.787	0.443	12
Cangdou 1817	0.617	0.000	0.691	0.547	9
Cangdou 1815	0.325	0.980	0.661	0.395	13
Cangdou 1814	0.286	0.440	0.352	0.350	14
Cangdou 1453	0.774	0.363	0.711	0.625	5
Cangdou 1438	1.000	0.840	0.752	0.898	1
Cangdou 1426	0.854	0.667	0.597	0.742	3
Cangdou 1418	0.803	0.656	0.627	0.720	4
Cangdou 1412	0.458	0.512	1.000	0.581	8
Cangdou 1327	0.781	0.438	0.314	0.592	6
Cangdou 1301	0.483	0.546	0.616	0.469	10
Cangdou 0734	0.746	0.857	0.000	0.751	2

including Cangdou 0734, 1453, 1817, 1418, 1426, 1327, 1412, and 1846), salt-alkali sensitive (five germplasms, including Cangdou 1821, 1819, 1301, 1815, and 1814), and highly salt-alkali sensitive (one germplasm, Cangdou 1850). Our results provide a basis for salt-alkali-tolerant soybean breeding and the development of salt-alkali land. In addition, principal component analysis of nine salt-alkali resistance evaluation indices was conducted for mature soybeans. Three indices, namely, hundred-grain weight, biomass, and yield, contributed 37.368% to the variability. These indices can serve as essential criteria for the rapid assessment of soybean salt-alkali tolerance.

Salt-alkali land is typically categorized as saline, alkaline, or mixed salt-alkali land containing multiple salt-alkali components (Tuyen et al. 2010). Previous research on salt-alkali tolerance identification of soybean germplasms has mainly focused on single neutral salts (e.g. NaCl) or alkaline salts (e.g., Na_2CO_3) (Shao et al. 1986; Ayers and Westcot 1989; Na et al. 2009). However, previous studies have indicated that mixed salt-alkali stress poses a more profound threat to plants than neutral or alkaline salt stress individually (Liu et al. 2018). Thus, in experiments conducted solely with single neutral or alkaline salts, it is difficult to comprehensively analyze the salt-alkali tolerance mechanism of soybeans and provide guidance for salt-alkali-resistant breeding. This issue is particularly relevant in China's coastal regions, where soil salinization is severe and often occurs alongside soil alkalization. The soil composition includes both alkaline and neutral salts (Xu and Luo 2012), with chloride salts and bicarbonate carbonates as significant salt-alkali components. Therefore, in the present study, experiments were conducted in open fields with four types of salts, including NaCl, Na_2CO_3, Na_2SO_4, and $NaHCO_3$, to accurately reflect the response of different soybean varieties to salt-alkali stress.

Conclusion

Salt-alkali tolerance is a complex trait of soybean germplasms. Therefore, a single index may not be sufficient for comprehensive evaluation of their salt-alkali resistance. However, when evaluating complex traits using multiple indices, inaccuracies can arise due to correlations

between these traits (Ayers and Westcot 1989; Tuyen et al. 2013; Guo et al. 2017; Zhang et al. 2018). The results of this study revealed strong correlations between the salt-alkali resistance coefficients of multiple indices. Furthermore, the rankings of the salt-alkali resistance coefficients for the individual indices varied among the different germplasms. These findings showed inherent limitations in evaluating the salt-alkali tolerance of soybean germplasms using single or multiple correlated indices. Therefore, in this study, salt-alkali resistance coefficients were adopted for various indices, and principal component analysis and stress tolerance assessment were employed to evaluate the salt-alkali resistance of 15 soybean germplasms. During data processing and resistance assessment, this approach minimized the overlap and crossover of representative information between the indices and considered the differences in the importance of comprehensive indices. This greatly enhanced the objective assessment of salt-alkali tolerance in soybean germplasms. Furthermore, this study established an evaluation model for salt-alkali resistance during soybean germplasm maturation through regression analysis and identified essential evaluation indices. Both models and indices provide a foundation for the rapid prediction of salt-alkali tolerance in other germplasms under similar conditions. Notably, in this study, only experiments related to salt-alkali stress during the maturation of 15 soybean germplasm lines were conducted, and the number of soybean germplasm lines was relatively limited; therefore, further research is warranted.

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Competing interests

The authors have declared that no competing interests exist.

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Supplementary material

Supplementary material 1

table S1: Biomass and yield of different soybean varieties under mild and severe salt-alkali stress; table S2: Correlation analysis for various salt-alkali tolerance indices; table S3: Coefficients and contribution rates of comprehensive indices for different indices.

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